A new diatom-based multimetric index to assess lake ecological status

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1   **A new diatom-based multimetric index to assess lake ecological status**

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7   **Abstract**

8   Eutrophication impairs lake ecosystems at a global scale. In this context, as benthic

9   microalgae are well-established warnings for a large range of stressors, particularly nutrient

10 enrichment, the Water Framework Directive required the development of diatom-based

11 methods to monitor lake eutrophication.

12 Here, we present the diatom-based index we developed for French lakes, named IBDL. Data

13 were collected in 93 lakes from 2015 to 2020. A challenge arose from the discontinuous

14 pressure gradient of our dataset, especially the low number of nutrient-impacted lakes. To

15 analyze the data we opted for the so-called "Threshold Indicator Taxa ANalysis" method,

16 which makes it possible to determine a list of "alert taxa". We obtained a multimetric index

17 based on different pressure gradients (Kjeldahl nitrogen, suspended matter, biological oxygen

18 demand and total phosphorous).

19 The IBDL proved to be particularly relevant as it has a twofold interest: an excellent

20 relationship with total phosphorus and possible application to any lake metatype. Its

21 complementarity with macrophyte-based indices moreover justifies the use of at least two

22 primary producer components for lake ecological status classification.

23 **Keywords**: ecological assessment, lakes, phytobenthos, Water Framework Directive

1-Introduction

Eutrophication is one of the most frequent consequences of human pressure on lake ecosystems at a global scale (Stenger-Kovacs et al., 2007). Primary producers are directly impacted since they are the base of the aquatic food web (Brauer et al., 2012). As the ability of species to compete differs according to nutrient availability, nutrient enrichment results in significant changes in community structure and function (Birk, 2012). For this reason, scientists and policymakers developed indices based on primary producer attributes to monitor eutrophication (Stevenson, 2014). In the early 2000s, the Water Framework Directive (WFD, 2000/06/EC) required all EU member states to implement bioassessment methods based, among other aspects, on the biological quality of "macrophytes and phytobenthos" to assess lake ecological status. This led to the development of numerous methods at the European level.

Poikane et al. (2016) reviewed this panel of methods and observed that countries generally developed separate assessment tools for macrophytes and phytobenthos, and that most of them considered diatoms, which are unicellular microalgae, to be a good proxy for phytobenthos. Diatoms are indeed early and well-established warnings for a large range of stressors, particularly nutrient enrichment (Stevenson, 2014). As a first step, indices originally dedicated to rivers were applied to lakes by the majority of member states (Kelly et al., 2014b), considering that many processes influencing diatom assemblages were comparable between lakeshores and shallow rivers (Cantonati and Lowe, 2014).

In some rare cases, diatom-based indices were developed specifically for lakes, based on species composition and abundance as for rivers (Bennion et al., 2014; Poikane et al., 2016). Diatoms from mud and silts were generally not considered, as they would respond to pore-water chemistry rather than water quality. The recommended sampling substrate varied

48 according to authors, from macrophytes to cobbles or even artificial substrates when no

49 natural substrates are found in all water bodies (King et al., 2006).

50 To harmonize the different national approaches, a European intercalibration exercise was

51 performed, involving eleven member states (Kelly et al., 2014b). France participated in this

52 exercise with the Biological Diatom Index (BDI, Coste et al., 2009), routinely used to assess

53 river ecological status. Although previous results tended to suggest there was a good

54 correlation between BDI and the environmental pressure gradients, at least in shallow lakes

55 (Cellamare et al., 2012), this intercalibration exercise revealed a poor correlation between

56 BDI values and total phosphorous across France (Kelly et al., 2014b). This was explained by

57 the absence of many lake taxa from the list of key species used to calculate the BDI, resulting

58 in an overall poor relevance of the final status assessment.

59 The aim of the present study was, therefore, to develop a new diatom-based index for lakes in

60 metropolitan France: the IBDL (*Indice Biologique Diatomées en Lac*: Diatom Biological

61 Index for Lakes). To collect the necessary data, we proposed a method (Morin et al., 2010)

62 consistent with a potential subsequent combination of this index with the existing French

63 macrophyte index IBML (*Indice Biologique Macrophytique en Lac*: Macrophyte Biological

64 Index for Lakes, Boutry et al., 2015). We detail here how diatom data were sampled and

65 analyzed and how we developed the IBDL. Finally, we discuss the relevance of this new

66 index, comparing the results obtained with index scores based on macrophytes, and assessing

67 its ability to reveal environmental gradients.

68 **2-Materials and Methods**

69 **2-1 Data collection**

70 Samples were collected from 93 French lakes (Figure 1) during the summer period, each year

71 from 2015 to 2020, according to Morin et al. (2010). The lakes were classified into three

72   metatypes based on alkalinity, according to the European intercalibration exercise previously

73   performed (Kelly et al., 2014b): low alkalinity (LA, alkalinity ≤ 0.2 meq.l^{-1}), medium

74   alkalinity (MA, 0.2 meq.l^{-1} < alkalinity < 1 meq.l^{-1}), and high alkalinity (HA, alkalinity ≥ 1

75   meq.l^{-1}). Diatoms were collected from both mineral substrates and lakeshore macrophyte

76   surfaces in observation units (OUs), whose number and location varied according to the lake

77   surface area and the riparian zone types. Such units are defined in the French macrophyte

78   sampling protocol for lakes NF T90-328 (AFNOR, 2022).
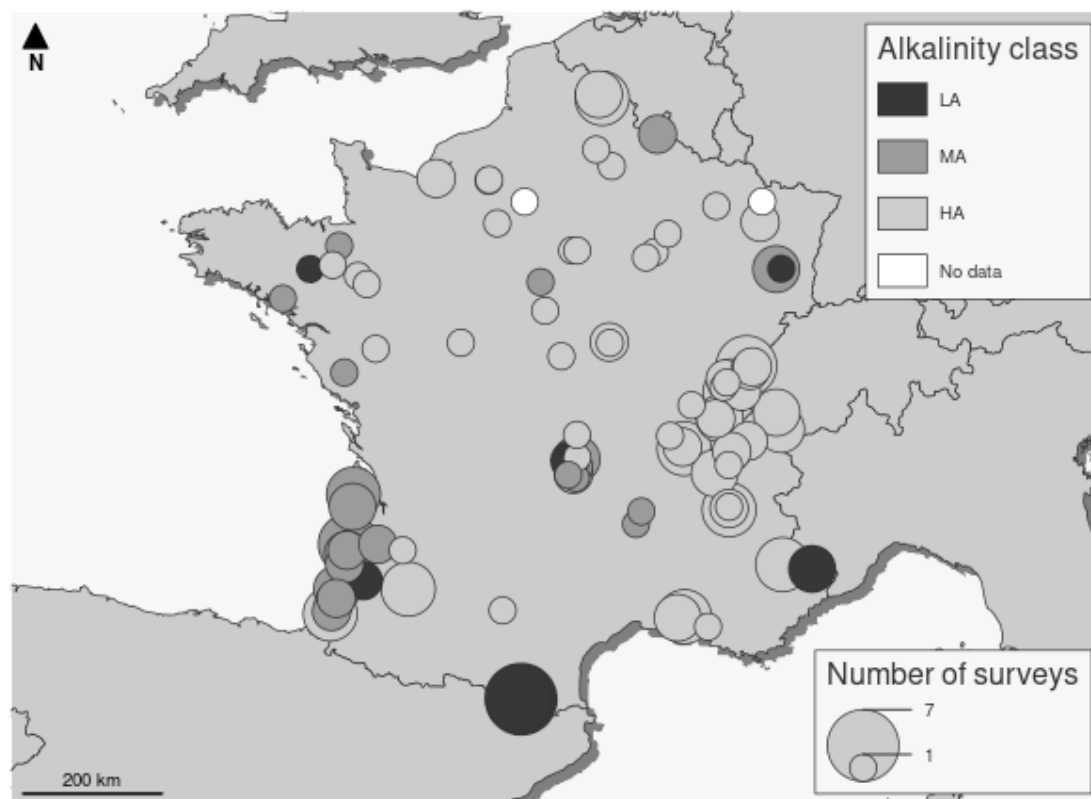

79

80   Figure 1: Study sites, number of surveys per site, and lake alkalinity classes (LA: low

81   alkalinity; MA: medium alkalinity; HA: high alkalinity) (Kelly et al., 2014a)

82

83   2-1-1 Biological data

84   Samples from hard mineral substrates were taken from at least five boulders or cobbles

85   selected at random for each OU. The total surface area sampled was equivalent to 100 cm², as

86   defined in the NF T90-354 standard (AFNOR, 2016). Selected substrates had to be submerged

87   within the euphotic zone, at a maximum depth of 0.5 m.

88   Samples performed on macrophytes were taken from helophytes (mainly *Phragmites australis*

89   (Cav.) Trin. ex Steud.). Green stem segments submerged for at least 4 to 6 weeks were

90   collected from a minimum of 5 macrophytes chosen at random. These stem segments had to

91   be located at a maximum depth of 0.2 m.

92   Diatoms were sampled from both substrates according to the NF T90-354 protocol, in line

93   with the European standards (EN 13946, European Commission). Cells were identified at

94   100x magnification by examining permanent slides of cleaned diatom frustules (400 valves

95   per slide) using, among others, Krammer and Lange-Bertalot (1986–1991) and Lange-

96   Bertalot (1995–2015, 2000–2013). Taxonomic homogenization was performed with Omnidia

97   6 software (Lecointe et al., 1993).

98   All OUs from a single lake were sampled within a maximum of 21 days. Diatom counts had

99   to include at least 350 cells per slide, with more than 50% of the diatom cells determined at

100   the species level, to comply with the NF T90-354 requirements.

101   2-1-2 Physico-chemical data

102   Parameter values were determined in summer at the deepest point of each lake, according to

103   European standards. Data were obtained from national surveillance monitoring programs.

104   Water quality analysis was not systematically performed each year: in a few cases, the most

105   recent physicochemical data available were collected three years before the diatom samples.

106   The following parameters were recorded: biological oxygen demand (BOD_5, mg.l^{-1}), oxygen

107   (O_2, mg.l^{-1}), oxygen saturation (% O_2), conductivity (Cond, μs.cm²), Kjeldahl nitrogen (NKJ,

108   mg.l^{-1}), ammonium (NH$_4$, mg.l^{-1}), nitrates (NO$_3$, mg.l^{-1}), nitrites (NO$_2$, mg.l^{-1}), orthophosphates

109   (PO$_4$, mg.l^{-1}), total phosphorous (Pt, mg.l^{-1}) and suspended particles (SP, mg.l^{-1}).

110   **2-2 Data analysis and index settlement**

111   All analyses were performed with R version 4.1.2 (2021-11-01) (R Core Team, 2021)

112   (Platform: x86_64-pc-linux-gnu (64-bit), Running under: Ubuntu 22.04.1 LTS).

113   Considering that the final dataset revealed a discontinuous trophic gradient, we opted for the

114   so-called "Threshold Indicator Taxa ANalysis" method (TITAN2 package, Baker et al.,

115   2020), which, based on bootstrapping and permutations, makes it possible to determine a list

116   of "alert taxa". The presence and/or increasing abundance of alert taxa reveal the existence of

117   anthropogenic pressures. TITAN replaces the community☐level response along a composite

118   gradient with taxon☐specific responses towards single environmental variables (Dufrêne and

119   Legendre 1997). Negative and positive responses are distinguished, and cumulative

120   decreasing or increasing responses in the community are tracked. This method is particularly

121   suitable for setting up multimetric indices.

122   A three-step procedure was necessary to build our Biological Diatom Index for Lakes (IBDL):

123   identification of alert taxa, choice of relevant metrics, and aggregation of these metrics to

124   obtain the final index score.

125   2-2-1 Identification of alert taxa

126   For the next part of the analysis, we set an occurrence threshold ≥3 for taxa to be included in

127   the index calculation (the so-called "index taxa").

128   TITAN combines change-point analysis (nCPA; King and Richardson 2003) and indicator

129   species analysis (IndVal, Dufrêne and Legendre 1997). Basically, the change-point analysis

130   compares within-group vs. between-group dissimilarity to detect shifts in community

131 structure along the environmental variable considered (for further details concerning this

132 method see Baker and King, 2010). Indicator species analysis then identifies the strength of

133 association between any particular taxon and this sample grouping. At the end of the process,

134 two IndVal scores are calculated for a single taxon in a two-group classification. The

135 algorithm finally classifies taxa into three different categories: Z^+ taxa, showing a significant

136 increase in abundance along the increasing environmental gradient; Z^- taxa, showing a

137 significant decrease along this gradient; and indifferent taxa, with no significant trend.

138 Alert taxa were defined as Z^+ or Z^- taxa whose shift thresholds were greater or lesser than the

139 community shift threshold.

140 2-2-2 Building metrics and selecting the relevant ones

141 For each environmental variable, a metric was calculated at the OU scale according to (1):

142 $$Metric_M = 1 - \left(\frac{Alert_{taxa}}{Index_{axa}}\right) \qquad (1)$$

143 Where:

144 $Alert_{taxa}$ is the number of alert taxa and $Index_{taxa}$ is the number of index taxa in the sample.

145 The metric value is bounded between 0 and 1. The lowest value (0) corresponds to a species

146 list entirely composed of alert taxa (determined for the environmental variable considered).

147 To build our index, we then selected the most relevant metrics, i.e., those with the best

148 relationship with the environmental parameter considered. We used Pearson's correlation

149 coefficients to measure this statistical association and only kept metrics showing a Pearson's

150 coefficient over 0.6. Metrics should significantly increase with impairment, significantly

151 decrease with impairment, or show no particular pattern. We obtained the response patterns of

152 the different metrics by transforming raw values into normalized deviations (Standardized

153 Effect Size: SES, Gotelli and McCabe, 2002; Mondy et al., 2012) (2). SES values made it

154   possible to obtain a single response pattern for a metric whatever the lake metatype and

155   substrate type considered.

156     $$SES_M = \left(\frac{Metric_M - M_{group}}{sd_{group}} \right) \qquad (2)$$

157   Where:

158     $Metric_M$ is the observed value of the metric, M_{group} and sd_{group} are the mean and standard

159   deviation, respectively, of the metric value for a given group of samples (i.e., substrate type x

160   lake alkalinity metatype) (values of M_{group} and sd_{group} are given in Table 1 S1)

161   The next step consisted of the normalization of SES values ($SESnor_M$) to make comparable

162   metric variation ranges (3):

163     $$SESnor_M = \frac{(SES_M - Min)}{(Max - Min)} \qquad (3)$$

164   Where:

165   SES_M is the observed value of SES for a given metric, Min its minimum value and Max its

166   maximum value in the whole dataset (values of Min and Max are given in Table 2 S1).

167   We further transformed metric values from normalized SES into the Ecological Quality Ratio

168   (EQR) (4), i.e. the ratio between the observed value of a metric ($SESnor_M$) and its expected

169   value under reference conditions (Kelly et al., 2014a), for any lake metatype and any substrate

170   ($SESnor_{Mref}$, values given in Table 3 S1).

171     $$EQR = \left(\frac{SESnor_M}{SESnor_{Mref}} \right) \qquad (4)$$

172

173   Finally, for each metric, we performed a Wilcoxon test to detect the potential influence of

174   substrate type on the EQR values obtained at the OU scale.

175   2-2-4 Aggregating metric values to obtain the final IBDL score

176   The final index score was obtained at the OU scale by averaging the selected metric values,

177   expressed in EQR.

178   For a score calculated for both mineral and macrophyte substrates, the lowest value was

179   considered the final score.

180   Each OU belongs to one of the four riparian zone types, as required in the NF T90-328

181   standard (AFNOR, 2022). These types were defined from the vegetation composition and/or

182   anthropogenic alterations of the lakeshore. The percentage of each riparian zone type was

183   estimated *in situ*, on the whole lake perimeter, during the sampling surveys. The final index

184   score for the whole lake was derived from a weighted average of the $Score_{OU}$ (5), taking into

185   account the percentage of the lake perimeter each OU represented in terms of riparian zone

186   type (Pc_{type}).

187    $$IBDL = \sum_{type=1}^{4}\left(\overline{Score_{OU}} * Pc_{type}\right) \qquad (5)$$

188   Finally, the resulting IBDL scores varied between 0 (worst water quality) and 1. Relationships

189   between IBDL scores and the different environmental variables considered were tested *a*

190   *posteriori* with simple linear regressions (R "mass" package, Venables & Ripley, 2002).

191

192   2-3 Comparing IBDL and IBML scores

193   We compared IBDL and IBML scores, based respectively on diatom and macrophyte

194   communities, to evaluate their complementarity or redundancy. IBML scores were computed

195   with the online application https://seee.eaufrance.fr/api/indicateurs/IBML/1.0.1 and the "httr"

196   package (Wickham, 2022).

197   We built a multiple linear regression model ("mass" package) to test which index correlated

198   best with Pt values: IBML, IBDL or a combination of both (mean value).

199

200   2-4 Preparing intercalibration

201   Considering a future intercalibration exercise, we analyzed the relationships between IBDL

202   scores and P_t for each lake metatype. A good correlation of the candidate metric with Pt

203   constitutes a key criterion for considering the index ready for integration into the

204   intercalibration process (Kelly et al., 2014b).

205   We also plotted IBDL against CM scores (intercalibration Common Metric, i.e., the Trophic

206   Index developed by Rott et al., 1998), to check their compliance. The CM was calculated with

207   Omnidia 6 software.

208

3-Results

210   Our data revealed discontinuous pressure gradients (Table 1), with a clear lack of impacted

211   conditions and an over-representation of lakes characterized by low eutrophication levels.

| Variable	% of missing values	mean	sd	median	p25	p75	maximum
ammonium (NH4, mg.l-1)	0.292	0.090	0.350	0.015	0.010	0.060	3.30
biological oxygen demand (DBO5, mg.l-1)	0.584	2.157	2.615	1.300	0.900	1.800	12.00
conductivity	0.309	230.108	124.368	243.500	158.000	297.000	815.00
Kjeldahl nitrogen (NKJ, mg.l-1)	0.292	0.661	0.959	0.250	0.250	0.700	6.90
nitrates (NO3, mg.l-1)	0.292	1.113	1.222	0.600	0.250	1.400	6.07
nitrites (NO2, mg.l-1)	0.292	0.011	0.025	0.005	0.005	0.010	0.30
orthophosphates (PO4, mg.l-1)	0.292	0.015	0.026	0.005	0.005	0.010	0.22
oxygen (O2, mg.l-1)	0.333	9.143	3.943	8.700	8.100	9.685	73.00
oxygen saturation (% O2)	0.333	110.203	20.910	108.000	101.000	117.650	187.00
suspended particles (SP, mg.l-1)	0.292	7.979	18.145	2.800	1.600	5.000	153.00
total phosphorous (Pt, mg.l-1)	0.292	0.027	0.067	0.005	0.005	0.015	0.51

212
213   Table 1. Physico-chemical data available for analysis. sd: standard deviation, p25: 25th

214   percentile; p75: 75th percentile

215 Biotic and abiotic data were obtained for 958 samples. Considering the data validation

216 criteria, 99% of the samples were included in the analysis. Data from both substrate types

217 were available for 552 OUs. Seven hundred eighty taxa were recorded, 8% of which were

218 identified to the genus level. One hundred and twenty-one alert taxa were determined out of

219 590 index taxa (S2).

220 We obtained the following Pearson test values for the different metrics at the OU scale: R = -

221 0.715 for the metric based on the parameter NKJ, R = -0.754 for BOD_5, R = -0.688 for Pt, R =

222 -0.666 for SP, R = -0.553 for PO_4, R = -0.329 for Conductivity, R = -0.174 for O_2, R = -0.265

223 for NO_2, and R = -0.204 for %O_2. Considering the selection rule proposed ($|R|>0.6$), only the

224 metrics based on NKJ, BOD_5, Pt and SP were considered to build the IBDL.

225 Metric values (in EQR) calculated from the lists of taxa sampled on mineral substrates and

226 macrophytes for a single OU did not differ significantly (p-value =0.65).

227 IBDL scores at the lake level were calculated from the selected metrics following the

228 aggregation rules proposed. The scores obtained were distributed as given in Figure 2. IBDL

229 could not be calculated for 20% of the samples due to incomplete floristic data.
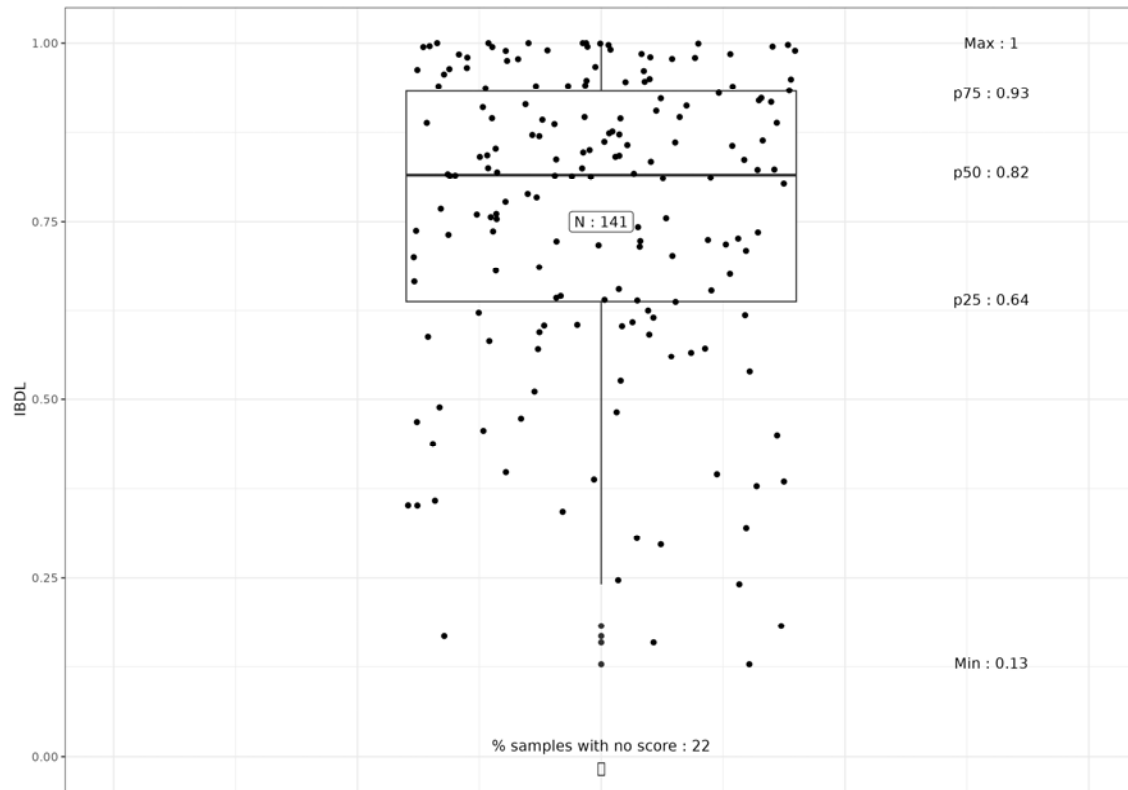
Figure 2: Distribution of the IBDL scores obtained (p25: 25th percentile; p50: median value; p75: 75th percentile).

The relationships between IBDL scores and the different environmental variables considered were very good (Figure 3) in both high-alkalinity and medium-alkalinity lakes. IBDL scores showed high correlations with these variables, particularly Pt, in both high alkalinity (R^2 = 0.63, p = $1.8e^{-15}$) and medium alkalinity lakes (R^2 = 0.83, p = $8.3e^{-11}$). Note that data from low alkalinity lakes were too scarce to perform such correlations.

238
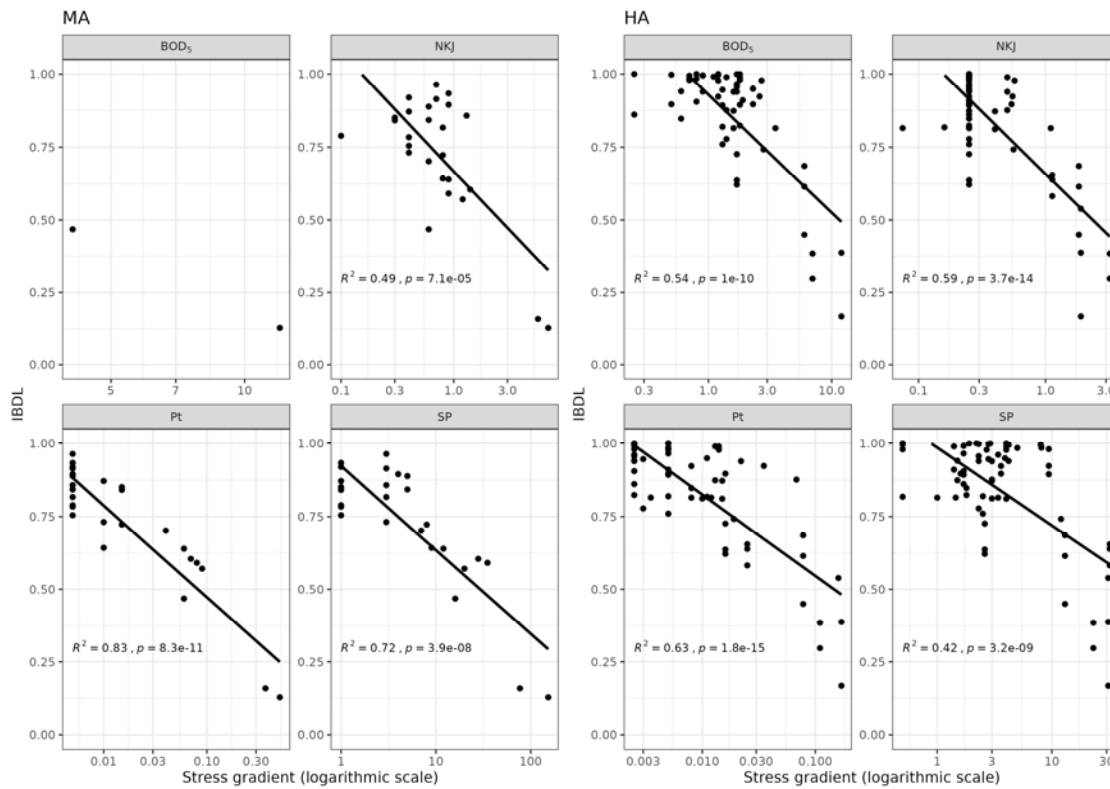
Figure 3. Relationships between IBDL and the environmental variables considered (MA: medium-alkalinity lakes; HA: high-alkalinity lakes; BOD_5: biological oxygen demand; NKJ: Kjeldahl nitrogen; Pt: total phosphorous; SP: suspended particles.

242

IBDL scores were also strongly associated with CM scores ($R2 = 0.52$ and $p = 2.2e^{-16}$ for high-alkalinity lakes; $R2 = 0.87$ and $p = 1.8\ e^{-7}$ for medium-alkalinity lakes) (Figure 4).
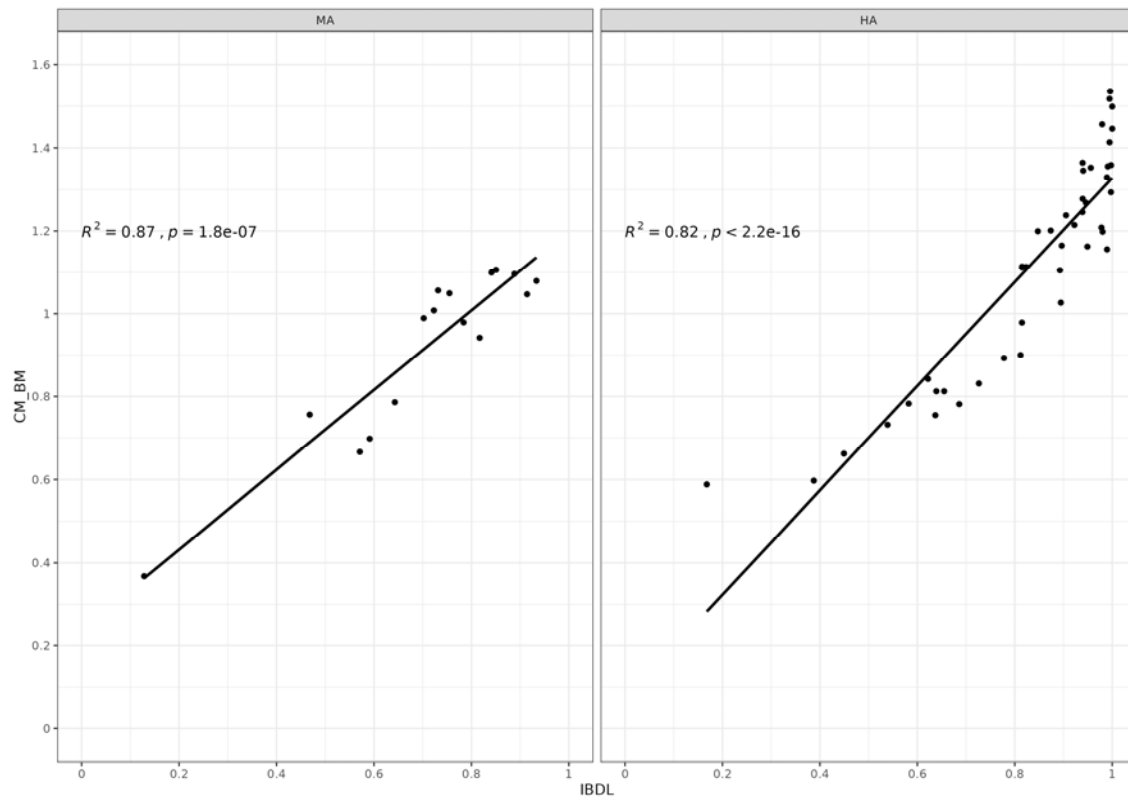
Figure 4: Relationships between IBDL and the common metric (CM) in medium alkalinity (MA) and high alkalinity (HA) lakes.

IBDL scores showed a better correlation with Pt (AIC = -171.44) than did IBML (AIC = -129.25) or a combination of both indices (AIC = -169.44). Nevertheless, IBDL tended to be generally less stringent than IBML (in 18 out of 22 samples), especially for scores higher than 0.8 (clearly dominant here). Figure 5 presents the difference between IBDL and IBML scores according to IBDL scores.
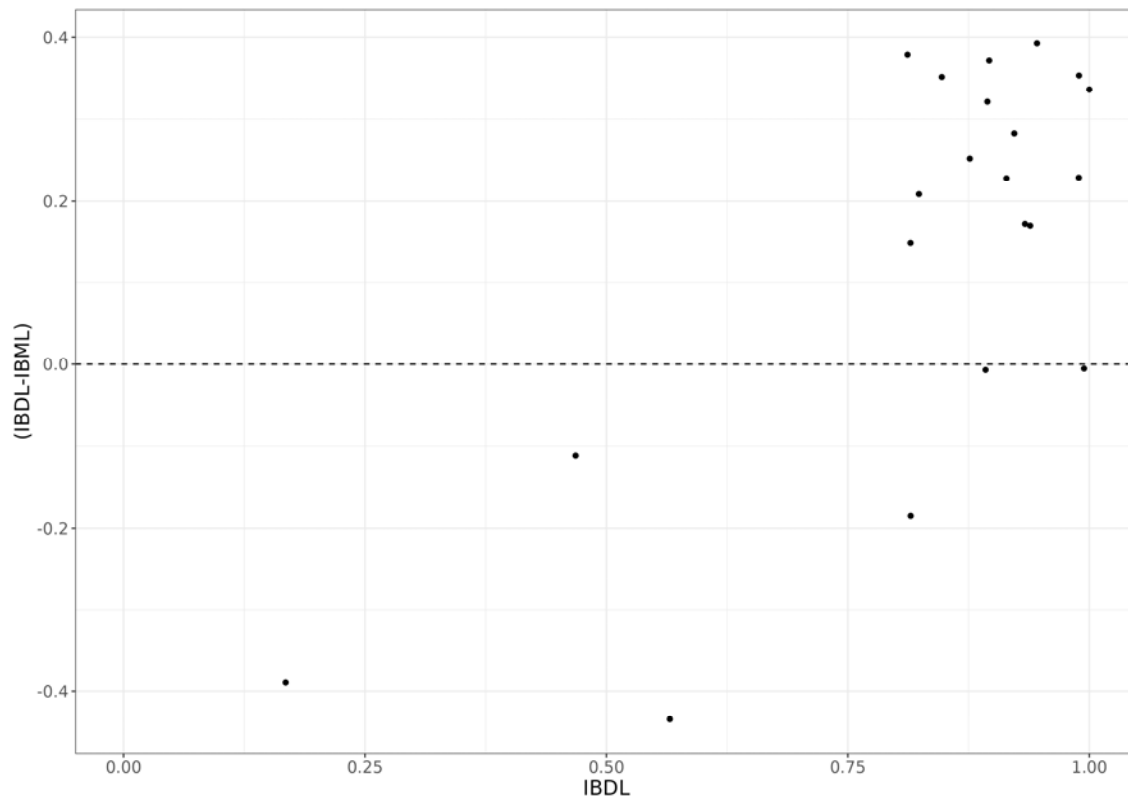
Figure 5: Difference between IBDL and IBML scores according to IBDL scores

4-Discussion

As required by the WFD, we developed a diatom index for the assessment of the ecological status of French lakes. We obtained very good correlations between IBDL and key environmental variables. One major challenge arose from the discontinuous pressure gradient of our dataset, especially the low available number of nutrient-impacted lakes.

The scarcity of impacted lakes in the datasets used to build diatom indices is not rare and has already been pointed out by some authors (Bennion et al., 2014). This lack makes it impossible to capture the entire trophic gradient or to build reliable species' ecological profiles. However, the majority of existing indices are calculated as an abundance-weighted average of the ecological profiles of every taxon from a sample, according to the Zelinka &

266   Marvan formula (Zelinka and Marvan, 1961). This method is far from optimal for datasets

267   showing discontinuous or very specific environmental conditions (Carayon et al., 2020). In

268   such cases, the identification of alert taxa seems more appropriate than considering diatom

269   communities as a whole. This has made the TITAN algorithm increasingly popular for

270   detecting specific taxa providing reliable signals of a specific stress (Khamis et al., 2014;

271   Costas et al., 2018; Gieswein et al., 2019; Carayon et al., 2020; Gonzalez-Paz et al., 2020).

272   Using this method, we built a multimetric index based on different pressure gradients (NKJ,

273   SP, BOD_5 and P_t). Although the strong influence of nutrients and organic matter on diatom

274   community composition is well established (Jüttner, 2010; Stevenson et al., 2013), diatom-

275   based metrics rarely take into account suspended particles for water quality assessment (but

276   see Larras et al., 2017). Diatoms are indeed directly impaired by turbidity, reducing light

277   availability for photosynthesis. Multimetric indices thus offer simple tools to summarize the

278   effect of multi-pressure gradients on communities (Riato et al., 2018), and can be considered

279   more effective for assessing biological conditions than a single metric (Stevenson et al.,

280   2013). However, despite their increasing use, multimetric indices suffer from the subjectivity

281   that can arise from metric selection (Reavie et al., 2008). Here, we attempted to avoid this

282   pitfall by proposing a method of selecting metrics based on the robustness of their response to

283   environmental gradients.

284   IBDL appears less stringent than IBML when assessing lakes' ecological status.

285   Literature comparing results from different indices in lakes, though scarce, tends to agree with

286   this overestimation of water quality by diatom-based methods (Kolada et al., 2016).

287   Phytobenthos has long been paid less attention than macrophytes for the assessment of lake

288   ecological status. It is true that recent diatom-based metrics barely detected newly impacted

289   lakes that would not have been detected by macrophyte metrics. Bennion et al. (2014)

290   showed, for example, that their index (LTDI) performed well for lakes with good ecological

291 status, but diatoms and other methods agreed less for lakes of lower status. This was

292 particularly the case in the presence of morphological alterations, for which diatoms are poor

293 indicators. A possible general explanation for the lower stringency of diatom-based indices in

294 lakes is the high abundance of species complexes like *Achnanthidium minutissimum* or

295 *Gomphonema parvulum*. Such complexes merge taxa that are morphologically close but with

296 different ecological preferences. Due to the existence of different taxa within the *A.*

297 *minutissimum* complex, many authors consider it an indicator of good water quality (Almeida

298 et al., 2014), whereas others consider it as tolerant towards toxic contaminants

299 (micropollutants) and hydrologic disturbances (Cantonati et al., 2014; Lainé et al., 2014).

300 Considering the generally high abundance of *A. minutissimum* in samples, this tends to blur

301 the overall pressure-response relationship between index scores and environmental variables

302 (Potapova and Hamilton, 2007). TITAN provides a means to avoid this pitfall, as such

303 complexes are not selected as alert taxa, given that their abundance dynamics do not show

304 clear response patterns to environmental gradients. Indeed, *A. minutissimum,* although highly

305 abundant in our dataset (22% of total species abundances), was not considered an alert taxon.

306 The fact remains that IBDL tends to be less stringent than IBML, despite better relationships

307 with Pt. In consequence, we have to explain why we think that the use of diatom-based

308 indices to assess lake ecological status is justified.

309 First, the discrepancy between macrophyte and diatom responses relies mainly on the

310 differences between their integration periods, given that indices provide information on

311 ecological conditions over the time an assemblage develops. Lavoie (2009) showed the

312 integration period of diatom-based indices to be about 2–5 weeks for nutrients, whereas

313 macrophytes react on yearly time scales (Kelly et al., 2016). As diatoms catch nutrients

314 directly from the water column (Wetzel, 2001), they also may be more directly sensitive to

315 rapid changes in trophic status than macrophytes (Vermaat et al., 2022). The rapid response of

316 phytobenthos should justify its routine use (Schneider et al., 2019), in particular for lakes in

317 non-equilibrium states (Kelly et al., 2016).

318 Second, diatom-based indices are essential where hydrologic pressures in littoral areas

319 prevent the development of macrophytes, and in lake typologies where macrophyte

320 communities are naturally species-poor or even absent (Schneider et al., 2019). Thus, while

321 macrophyte-based indices cannot be calculated in all lakes, this is not true for diatom-based

322 indices. Moreover, our results show that, with IBDL, water quality managers can directly

323 compare ecological status assessments from different lakes even if the substrate sampled is

324 different. Many studies highlighted that allelopathic relationships between macrophytes and

325 epiphytic diatoms may be responsible for specific associations between macrophytes and

326 diatom species and, thus, may contribute to the organization of particular assembly patterns

327 (Hinojosa-Garro et al., 2010). In any case, in terms of ecological preferences, and

328 consequently in terms of IBDL scores, our results did not show any significant differences

329 between communities sampled on mineral substrates or macrophytes at the OU level,

330 corroborating previous results obtained by Kitner and Poulíčková (2003) and Bennion et al.

331 (2014). Other studies even support the use of epiphytic diatoms as biological indicators for

332 lakes irrespective of the dominant macrophyte species sampled (Cejudo-Figueiras et al.,

333 2010). The key point is to avoid senescent material or recently grown shoots that would

334 potentially induce a colonization stage effect (King et al., 2006).

335 The next challenge was to check the consistency of the resulting classification of lakes

336 based on IBDL to the harmonized definition of good ecological status established in the

337 completed intercalibration exercise (Kelly et al., 2014b). The first step consisted in testing the

338 correlation between IBDL scores and total phosphorus in our dataset. Only HA and MA

339 typologies were considered here but, in any case, the last intercalibration exercise could not

340 be performed for LA lakes. We obtained very good correlations that are clearly an

341   improvement compared to the non-significant relationship previously obtained between BDI

342   (diatom index used for the assessment of rivers) and Pt, and even better than the pressure-

343   impact relationships observed at a pan-European scale (R^2 between national methods and Pt

344   ranged from 0.32 to 0.66 max., Kelly et al., 2014b). The second step consisted in testing the

345   correlation between IBDL scores and the intercalibration common metric (CM) scores, in

346   EQR. Here, the correlations demonstrated a very good agreement between IBDL and CM

347   scores in both medium ($R^2 = 0.87$) and high alkalinity ($R^2 = 0.82$) lakes. We are, therefore,

348   confident in our ability to match IBDL ecological status thresholds with those validated at the

349   European level.

350   **Conclusion**

351   The new diatom index proposed here meets the requirements of the WFD and makes it

352   possible to assess lakes' ecological status in metropolitan France. The IBDL has proved to be

353   particularly relevant as it has a twofold interest: an excellent relationship with total

354   phosphorus and an application in any lake metatype. Its complementarity with IBML justifies

355   the use of at least two primary producer components for ecological status classification (Kelly

356   et al., 2016).

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361   **Author's contributions**

362   All authors participated in designing the study and developing aims and research questions.

363   S.B. designed methodology, extracted data and made the analyses, supported by T.L.

364   concerning pretreatments before intercalibration. J.T.R. led the writing of the manuscript

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366   to the final version of the manuscript, and gave final approval for publication.

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